HPLC-SPE-NMR for combinatorial biosynthetic investigations – expanding the landscape of diterpene structural diversity

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Publication date:
2016

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HPLC-SPE-NMR for combinatorial biosynthetic investigations – Expanding the landscape of diterpene structural diversity

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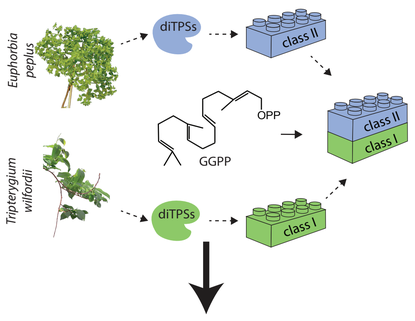
Significance

In this work, the analytical technique, HPLC-HRMS-SPE-NMR[1] was used for the first time in combination with combinatorial biosynthetic investigations in *N. benthamiana*. This efficient setup allowed for identification of several diterpene synthase (diTPS) combinations responsible for stereospecific biosynthesis of high-value natural products and stereoisomers.

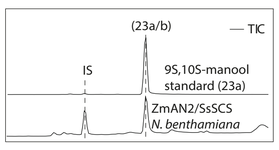
Method

diTPS combinations infiltrated in N. *benthamiana* were screened by GC-MS followed by HPLC-HRMS-SPE-NMR analysis of selected metabolites for structural clarification.

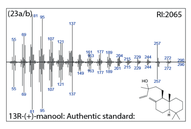


Gene expression

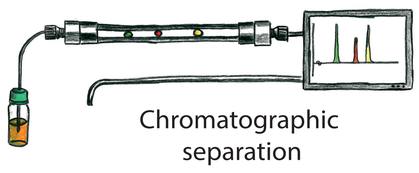
GC-MS screening



Comparison with standards

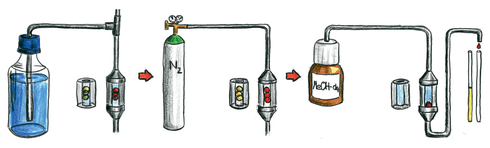


HPLC-HRMS



Chromatographic separation

SPE trapping



NMR analysis

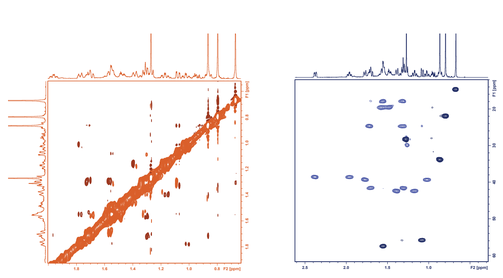
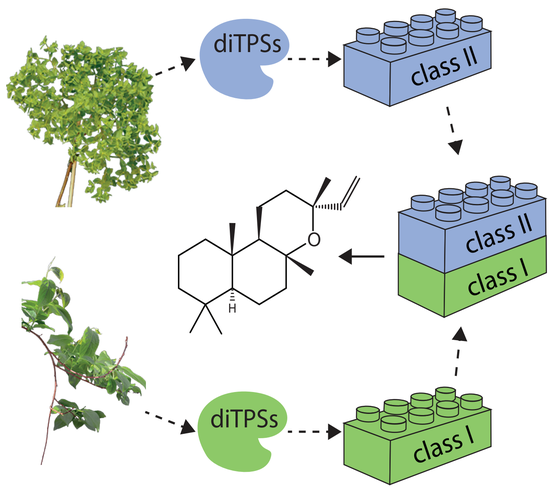


Figure 1. Methodology behind the combinatorial biochemical investigations.

Background

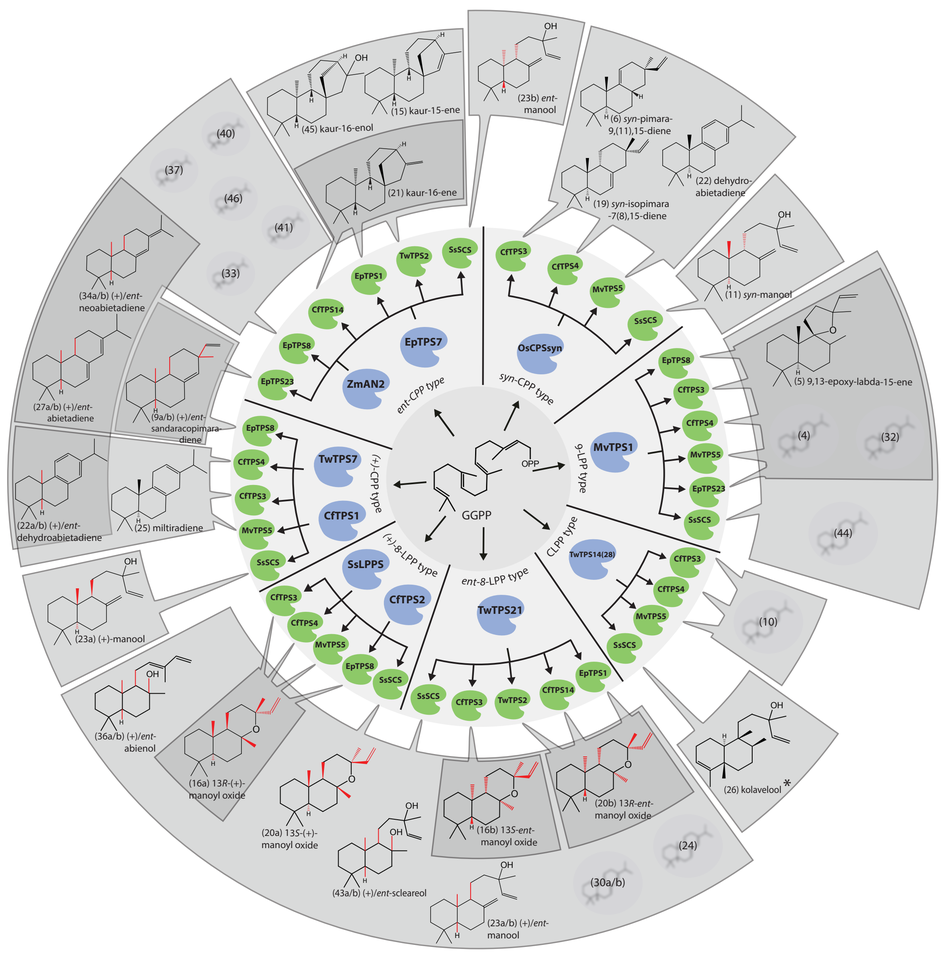
Diterpenoids are a diverse class of structurally complex natural products with broad therapeutic applications and and high commercial value. Exploitation, however, is limited by low natural abundance and complex synthetic routes.

Biosynthesis of diterpenoids is a two-step enzyme reaction by a pair of class I and class II diTPS (or 1 bifunctional diTPS).[2] With proper knowledge of individual diTPS products, biosynthetic production in e.g, yeast is a promising, biosustainable alternative, which has been investigated by a combinatorial approach.



Results

51 functional combinations of class I and II diterpene synthases were constructed as depicted in the combinatorial wheel below.[3] These led to stereoselective biosynthesis of over 50 diterpene skeletons, including natural variants and novel enantiomeric or diastereomeric counterparts such as manool (**11**, **23a**, **23b**) and manoyl oxide (**16a**, **16b**, **20a**, **20b**) analogues.



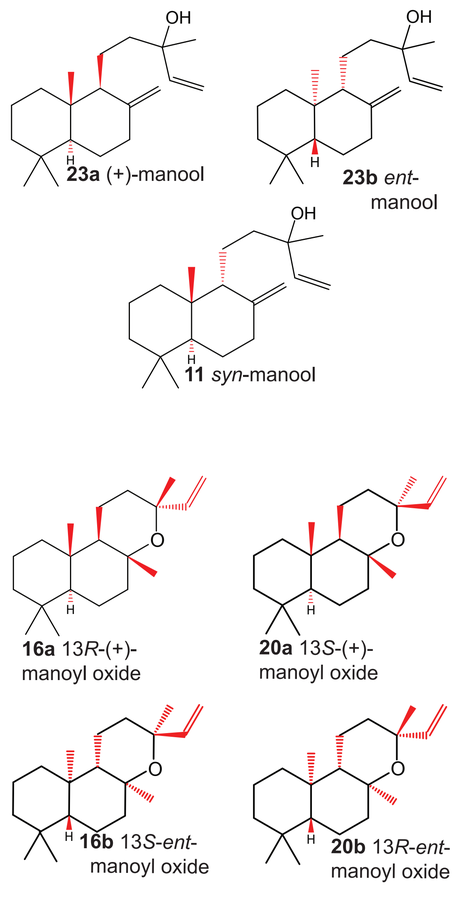


Figure 2. Combinatorial wheel showing formation of diterpenes by diTPS combinations including the stereochemically controlled manool and manoyl oxide analogues

Due to lack of suitable functional groups and epimerization of the manool C13, combined information from ^{1}H chemical shifts, optical rotation, chiral GC, and knowledge of stereospecificity of class II diTPS were used for assignment of stereochemistry.

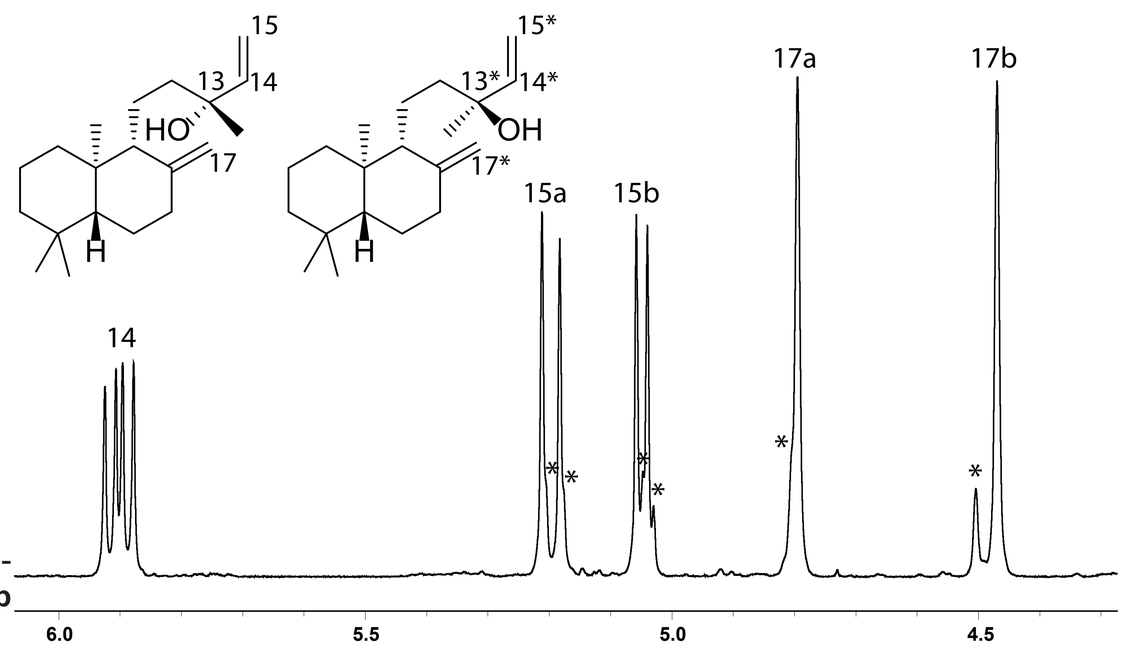


Figure 3. C13 epimer resonances of **23b** indicated by asterisks

References

[1] Johansen, Wubshet, Nyberg, Jaroszewski. *J. Nat. Prod.* **2011,** 74, 2454–2461. [2] Zerbe, Bohlmann. *Trends Biotechnol.* **2015**, 33, 419–428. [3] Andersen-Ranberg, Kongstad, Nielsen, Jensen, Pateraki, Bach, Hamberger, Zerbe, Staerk, Bohlmann, Møller, Hamberger. *Angew. Chem. Int. Ed.* **2016,** doi: 10.1002/anie.201510650